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STUDIES ON BIOACTIVITY OF *CHARA BRAUNII* C.C. GMELIN (*CHAROPHYTA*)

Chara braunii a freshwater green algae belonging to the division *Charophyta* was collected from different freshwater habitats of Faisalabad region of Pakistan. Its antifungal activity was determined by using agar well diffusion method against ten fungal cultures. The results showed a significant response and promising antifungal activity of *Ch. braunii* against the tested fungal species. The present research work is highly significant in terms of understanding the response of important new natural sources of bioactive compounds for pharmaceutical industry and it is first comprehensive report from Faisalabad region.

Key words: algae, *Charophyta*, *Chara*, bioactivity.

Introduction

As producers of diverse arrangement of toxic and other active compounds which are biologically active with abundant properties in biomedicine, algae have received attention (Naila et al., 2005; Ghazala et al., 2005; Shahnaz et al., 2006; et al., 2010; Khalid, Shameel, 2011a, b; 2012a–d). Freshwater algae play a very important role in aquatic system. *Chara braunii* is green algae of fresh water belonging to the division *Charophyta*. Distribution of *Ch. braunii* is all over the world (Imahori, 1954; Kasaki, 1964; Wood and Imahori, 1965). This species is monoecious and by the absence of cortex, easily distinguished by other species of chara. Morphology includes the stipulodes developing at the base of whorled branchlets (Wood, Imahori, 1965). There are variations in the size of its thalli and oospores. It has wider environmental tolerance, because not only exist in the bottom of deep lakes but also in the shallow water areas (Imahori, 1954; Wood, Imahori, 1965). The main objective of this study was to find new natural sources of bioactive compounds for pharmaceutical industry to facilitate the nation.

Materials and methods

Collection sites. Faisalabad is the major and third largest city in Pakistan, the second largest in the province of Punjab after Lahore. In the heart of Pakistan it is the major industrial centre. There are different small and large freshwater bodies such as canals, small streams and ponds present here and there from which *Ch. braunii* where collected.

Algal material. The collections of algal material were made during September 2011 to April 2012. In order to get the maximum amount of algae for better results, to collect the same species repeated attempts were made regularly.

Some free floating algae were collected from the top surfaces and attached algae from the edges, walls of canals, ponds, close to the bottom and slow running water bodies. The collected material were brought to the lab and to remove the litter, sand particles, aerophytes, external parasitic organisms, animal dung and attached dust, washed thoroughly. To prevent the breakdown of large molecules of certain thermo labile natural products, the main mass of the material was dried in shade. Then the dried mass of algae was chopped into small pieces and weighed.

Antifungal activity. The ten fungal species were used for antifungal bioanalysis i.e. *Aspergillus flavus*, *Fusarium moniliforme*, *F. oxysporum*, *Microsporium canis*, *Phythium aphanidermatum*, *Ph. oedocephalum*, *Rhizoctonia solani*, *Trichoderma hamatum*, *T. harzianum* and *Trichophyton longifusus*. Antifungal activity was determined by using agar well diffusion method against mentioned fungal cultures. The algal extraction and procedures for different tests of bioactivity have already been described in detail (Khalid et al., 2010).

This is performed by agar tube dilution; the protocol of Brass et al. (1979) for *in vitro* fungicidal bioassay is as follows:

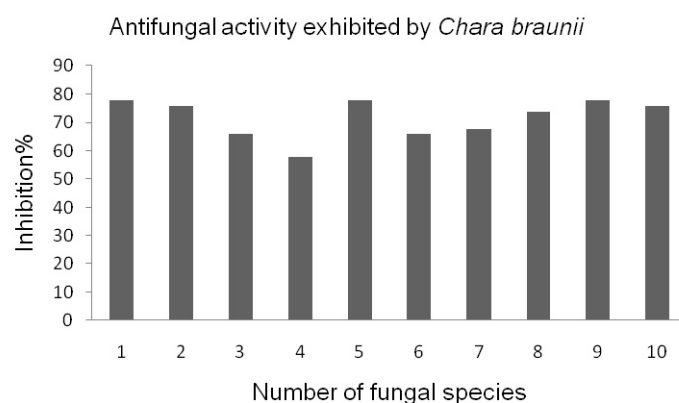
- Methanol extract of the alga was dissolved in sterile DMSO, serving as stock solution.
- Sabouraud agar was prepared by mixing Sabouraud 4 % glucose agar and of agar-agar in 500 mL × distilled water.
- It was then steamed to dissolve and dispensed a known amount into screw capped tubes.
- Tubes containing medium were autoclaved at 121 °C for 15 minutes.
- The tubes were allowed to cool to 50 °C, and nonsolidified Sabouraud agar medium were poisoned with 200 µL of extract pipetted from the stock solution. This gave final concentration of 200 µg/mL of medium and 400 µg/mL of medium for pure and crude extracts respectively.
- Tubes were then allowed to solidify in slanted position at room temperature.
- Each tube was inoculated with a 4 mm diameter piece of inoculum removed from a 7 day old culture of fungi.
- For nonmycelial growth an agar surface streak was employed.
- Other media were supplemented with DMSO to serve as control.

Results

Chara braunii showed a significant response and strong antifungal activity against *Aspergillus flavus*, *Phythium aphanidermatum*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichophyton longifusus* and *Fusarium moniliforme*. Among all these fungi, *Ch. braunii* showed most promising results against *T. harzianum*, *Aspergillus flavus* and *Phythium aphanidermatum* and this alga showed poor results against *Microsporium canis* (Table, Figure).

Antifungal activity exhibited by *Chara braunii*

Fungal culture	Inhibition, %
<i>Aspergillus flavus</i>	78
<i>Fusarium moniliforme</i>	76
<i>F. oxysporum</i>	66
<i>Microsporium canis</i>	58
<i>Phythium aphanidermatum</i>	78
<i>Ph. oedoehilum</i>	66
<i>Rhizoctonia solani</i>	68
<i>Trichoderma hamatum</i>	74
<i>T. harzianum</i>	78
<i>Trichophyton longifusus</i>	76



Discussion

There are different small and large water bodies such as canals and ponds etc. present here and there in Faisalabad from which *Ch. braunii* were collected. It is found in the Baltic Sea and also in fresh water (Zhakova, 2003). It's requiring only shallow and sheltered habitat. Mostly appears in the bottoms. However, more often it is found in fish ponds and rice fields, growing there as weeds (Kasaki, 1964). Morphological variability and intraspecific phylogeny of ubiquitous species *Ch. braunii* reported from Japan (Kato et al., 2008). This species is multicellular and has resemblance with land plants because it has stem and leaf-like structures. Through the rhizoids branching system is attached with the substrate. Cell wall is made up of calcium salt therefore very rough to touch. This species has been classified into seven forms and on the

bases of morphology of oospores, bracts, stipulodes and so on, these seven forms are distributed from one another (Wood, Imahori, 1965).

Chara braunii showed strong antifungal activity against *Aspergillus flavus*, *Phythium aphanidermatum*, *Trichoderma harzianum*, *T. hamatum*, *Trichophyton longifusus* and *Fusarium moniliforme*. Among all these fungi, it showed most promising results against *Trichoderma harzianum*, *Aspergillus flavus* and *Phythium aphanidermatum* and this alga showed poor results against *Microsporum canis* (Table, Figure). It was first time reported from Faisalabad region of Punjab province of Pakistan. The main objective of this study was to find new natural sources of bioactive compounds for pharmaceutical industry.

The species produce a variety of bioactive metabolites that may have allelochemical functions in the natural environment, such as in the prevention of fouling by colonizing organisms. Their chemical compounds are of biotechnological interest, especially for clinical immunosuppressive and enzyme inhibiting activities. Their metabolites have the potential for use in antifouling technology, since they show antibacterial, antifungal and antimacroufouling properties which could be exploited in the prevention of biofouling on man-made substrata in the aquatic environments (Dahms et al., 2006).

The present research work highly significance in terms of understanding the response of important new natural sources of bioactive compounds for pharmaceutical industry. It is first comprehensive report from Faisalabad region.

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ИЗУЧЕНИЕ БИОЛОГИЧЕСКОЙ АКТИВНОСТИ *CHARA BRAUNII* С.С. GMELIN (*CHAROPHYTA*)

Chara braunii – пресноводный вид водорослей из отдела *Charophyta*, был собран в различных пресноводных местообитаниях Фейсалабадской области Пакистана. Антигрибковую активность этого вида изучали методом диффузии на агаре культур 10 штаммов грибов. Установлено влияние *Ch. braunii* на исследуемые культуры, свидетельствующее об ее антифунгальном характере. Результаты исследования важны для поиска новых природных источников биоактивных веществ, применяемых в фармацевтической промышленности. Это первое подобное исследование в регионе.

К л ю ч е в ы е с л о в а : водоросли, *Charophyta braunii*, *Chara*, биологически активные вещества.